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REVIEW

Donor Cell Leukemia: A Review

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Relapse of acute leukemia following hematopoietic stem cell transplantation (HSCT) usually represents return of an original disease clone, having evaded eradication by pretransplant chemo-/radiotherapy, conditioning, or posttransplant graft-versus-leukemia (GVL) effect. Rarely, acute leukemia can develop de novo in engrafted cells of donor origin. Donor cell leukemia (DCL) was first recognized in 1971, but for many years, the paucity of reported cases suggested it to be a rare phenomenon. However, in recent years, an upsurge in reported cases (in parallel with advances in molecular chimerism monitoring) suggest that it may be significantly more common than previously appreciated; emerging evidence suggests that DCL might represent up to 5% of all posttransplant leukemia "relapses." Recognition of DCL is important for several reasons. Donor-derivation of the leukemic clone has implications when selecting appropriate therapy, because seeking to enhance an allogeneic GVL effect would intuitively not have the same role as in standard recipient-derived relapses. There are also broader implications for donor selection and workup, particularly given the growing popularity of nonmyeloblative HSCT and corresponding rising age of the potential donor pool. Identification of DCL raises potential concerns over future health of the donor, posing ethical dilemmas regarding responsibilities toward donor notification (particularly in the context of cord blood transplantation). The entity of DCL is also of research interest, because it might provide a unique human model for studying the mechanisms of leukemogenesis in vivo. This review presents and collates all reported cases of DCL, and discusses the various strategies, controversies, and pitfalls when investigating origin of posttransplant relapse. Putative etiologic factors and mechanisms are proposed, and attempts made to address the difficult ethical questions posed by discovery of donor-derived malignancy within a HSCT recipient.

Biol Blood Marrow Transplant 17: 771-789 (2011) © 2011 American Society for Blood and Marrow Transplantation

KEY WORDS: Donor, Acute leukemia, Hematopoietic stem cell transplantation, Leukemogenesis

INTRODUCTION

Secondary malignancy is an important cause of morbidity in transplantation practice, but its occurrence in tissues of donor origin is rare. One study of 108,062 solid-organ transplants detected 21 examples, for a reported incidence of 0.017% [1]. Allogeneic hematopoeitic stem cell transplantation (HSCT) is an effective treatment for many hematologic malignancies, but disease relapse remains a major cause of posttransplant mortality [2]. Most cases represent recurrence of original disease through outgrowth of residual cells having evaded eradication by conditioning or graft-versus-disease effect. Occasionally, relapsed disease may display different phenotypic or cytogenetic features from the original disease. This may be because of a lineage switch (eg, myeloid to lymphoid blast crisis), clonal evolution, or emergence of latent surviving subclones [3], but usually the relapse clone is host derived. Rarely, acute leukemia can develop de novo in donor-derived cells. Reporting of so-called "donor cell leukemia" (DCL) has accelerated in recent years, and this intriguing entity is the subject of considerable interest for the unique insights into the mechanisms of leukemogenesis it might provide.

Incidence of DCL

DCL was first described by Fialkow and colleagues in 1971 [4]. A 16-year-old female patient with acute lymphoblastic leukemia (ALL) relapsed 62 days after bone marrow transplant (BMT) from an HLA-identical brother. Cytogenetics at relapse revealed only male (XY) metaphases, leading the authors to conclude that leukemic transformation had occurred in progeny of engrafted donor bone marrow (BM) cells. They implicated activation of a leukemogenic agent in susceptible donor cells in this process. Further cases have been reported sporadically since, mostly as isolated reports and small series. Efforts to estimate

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Financial disclosure: See Acknowledgments on page 785.
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Received September 20, 2010; accepted October 8, 2010

1083-8791/$36.00
doi:10.1016/j.bbmt.2010.10.010

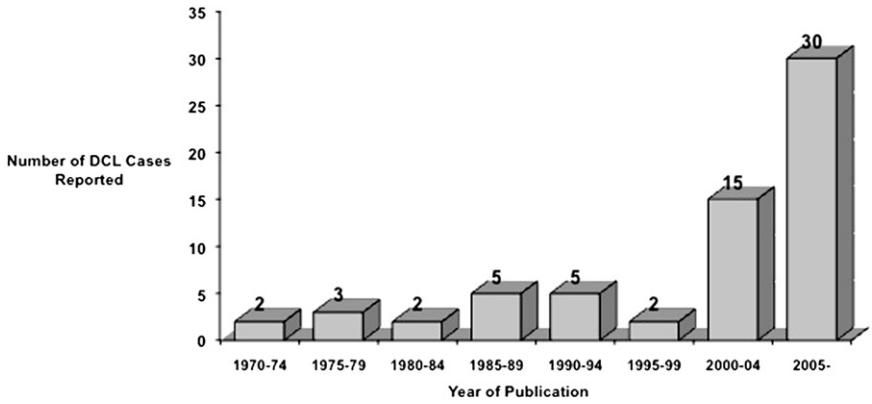

Figure 1. Number of reported cases of donor cell leukemia (DCL) by year of publication (1971-2010).

the incidence of DCL are hampered by the sporadic nature of reports and habitual difficulties in confirming the diagnosis. In 1982, Boyd et al. [5] suggested that DCL might account for ≥5% of posttransplant leukemia relapses, although for many years, the paucity of reports suggested this to be a gross overestimate. Recently, however, frequency of reporting has accelerated with more cases reported since 2004 than during the previous 34 years (Figure 1). In 2005, a retrospective survey by the European Group for Blood and Marrow Transplantation (EBMT) asked all registered centers to report any cases of suspected or proven DCL encountered at their institutions, identifying 14 cases [6]. Because the 91 responding centers had performed a total 10,489 procedures, DCL incidence was estimated at 124 per 100,000 transplants. Most occurred within 4 years of HSCT, suggesting an annual incidence greatly exceeding the background incidence of acute leukemia. In 2006, Salla-Torra et al. [7] reported 6 cases identified at their institutions since 1974, and Ruiz-Argüelles et al. [8] prospectively demonstrated donor origin in 2 of 40 consecutive relapses for a putative incidence of 5%: a figure intriguingly similar to that proposed by Boyd 24 years earlier.

Using various PubMed searches, we have identified 51 cases of suspected or proven DCL in the English literature, along with 13 cases of donor cell–derived myelodysplastic syndrome (DCM) lacking evidence of transformation to frank leukemia at time of publication [4,6-57] (Table S1). DCM cases with evidence of clonality, high-risk features, or progression toward acute myelogenous leukemia (AML) were included in the cohort; these probably share comparable epidemiologic and biological features with other DCLs and represent the same phenomenon detected at an earlier stage, supported by the observation that at least 11 cases of *frank* DCL apparently displayed an antecedent myelodysplastic phase.

DCL Cohort Characteristics

Patients reported to have developed DCL appear to be a heterogeneous cohort, with characteristics and transplant details broadly reflecting those for the general HSCT population. Summary characteristics are presented in Table 1. Median age at transplantation of patients developing DCL was 31.0 years (range: 1–61), with 18 (29%) aged ≤16 years and no discernable sex preponderance (32 male/32 female). Patients who received myeloablative (MA) conditioning were younger than those receiving nonmyeloablative (NMA) conditioning (29.5 versus 40 years). Intuitively, donor age might influence the risk of leukemia developing in their donated HSCs. Median donor age (in non-cord blood transplants) was 22.0 years, but this information was only provided in 17 of the 60 reports. Where both ages were provided, donors were older than recipients in 9 (of 16) cases, by an average of 3.6 years. Donors were female in 34 (53%) and male in 30 (47%), with recipient/donor sex mismatch present in 40 (63%) cases (including 17 of the 20 earliest reports).

Primary diagnosis was chronic myeloid leukemia (CML) in 17 (26%) cases, including 2 in lymphoid blast crisis; AML in 10 (16%); ALL in 17 (26%); and myelodysplastic syndrome (MDS) in 3 (5%), including a therapy-related MDS after treatment for chronic lymphocytic leukemia (CLL). Seven patients (11%) were transplanted for aplastic anemia (AA). Single reports describe DCL after juvenile myelomonocytic leukemia, myeloid/natural killer cell precursor acute leukemia, multiple myeloma, follicular lymphoma, Hodgkin lymphoma, adult T cell lymphoma/leukemia (ATL), beta-thalassemia, Langerhan's cell histiocytosis, and renal cell carcinoma. We recently observed a case of DCL following HSCT for primary (aleukemic) granulocytic sarcoma. DCL type was AML in 34 (53%) cases and ALL in 16 (25%). Another was described as CML in lymphoid blast crisis. DCL differed from original disease in 46 (72%) cases, with demonstrable phenotypic, cytogenetic, or subclassification differences present in many others. In addition to 13 DCM cases, an antecedent MDS phase was diagnosed in at least 11 of the 34 donor-derived AML cases (32%). Others might have also followed an MDS

Table 1. Patient, Donor, and Transplant Characteristics for the 64 Reported Cases of Donor Cell Leukemia

Patient Characteristics		
Sex		Data Missing
Male	32 (50%)	0
Female	32 (50%)	
Age at transplant (median 31.0 years)		
<10	10 (16%)	1
10-19	10 (16%)	
20-29	9 (14%)	
30-39	16 (25%)	
40-49	13 (21%)	
≥50	5 (8%)	
Primary diagnosis		
AML	10 (16%)	0
ALL	17 (26%)	
CML	17 (26%)	
MDS	3 (5%)	
AA	7 (11%)	
Other (single cases)	10 (16%)	
DCL type		
AML	34 (53%)	0
ALL	16 (25%)	
MDS	13 (20%)	
Other	1 (2%)	
Donor characteristics		
Donor		
Sibling	47 (74%)	0
Other relative	4 (6%)	
VUD	9 (14%)	
Cord	4 (6%)	
Sex		
Male	30 (47%)	0
Female	34 (53%)	
Age at donation (median 22.0 years)		
<10	4 (23%)	43
10-19	4 (23%)	
20-29	2 (12%)	
30-39	3 (18%)	
40-49	4 (24%)	
≥50	0	
Transplant details		
HSC source		
BM	45 (70%)	0
PB	15 (24%)	
CB	4 (6%)	
Conditioning		
Myeloablative	57 (89%)	0
Nonmyeloablative	7 (11%)	
TBI with CP (±other drugs)	26 (44%)	3
TBI without CP (±others)	7 (11%)	
TLI/TAI with CP (±others)	2 (3%)	
CP + BU (±other drugs)	16 (26%)	
CP (±other drugs)	8 (13%)	
Other	2 (3%)	
Alkylating agent	55 (90%)	
Topoisomerase II inhibitor	20 (33%)	
Irradiation (TBI/TLI/TAI}	35 (57%)	
T-depletion of graft		
Ex vivo	4 (*)	*
In vivo (ATG/alemtuzumab)	10 (*)	
GVHD prophylaxis		
CSA with MTX (±others)	31 (57%)	10
CSA without MTX (±others)	13 (24%)	
MTX without CSA (±others)	9 (17%)	
Other	1 (2%)	
CMV reactivation	8 (*)	*
Chronic GVHD	23 (*)	*
Extensive	7 (*)	

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; AA, aplastic anemia; VUD, volunteer unrelated donor; HSC, hematopoietic stem cell; BM, bone marrow; PB, peripheral blood; CB, cord blood; TBI, total-body irradiation; CP, cyclophosphamide; TLI, total lymphoid irradiation; TAI, thoracoabdominal irradiation; BU, busulfan; ATG, antithymocyte globulin; GVHD, graft-versus-host disease; CSA, cyclosporine; MTX, methotrexate; CMV, cytomegalovirus.
*Details rarely/inconsistently provided in published reports.

prophase that either evaded detection or was omitted from the published report. Patients displaying an MDS phase were significantly older (median 36 versus 27 years), perhaps suggesting that DCL might represent a convergent endpoint of distinct mechanisms of leukemogenesis. Forty-six percent of cases documenting donor MDS had transformed to frank AML at time of publication, but this apparently high transformation rate might simply reflect a higher sensitivity of detecting antecedant MDS, given the intensive monitoring afforded to patients after HSCT.

The donor was related to the recipient in 80% of cases (47 siblings; 1 HLA-identical mother; 3 other haploidentical relatives). Only 9 (14%) involved a volunteer unrelated donor (VUD). HSC source was BM in 45 cases (70%), peripheral blood (PB) in 15 (24%), and cord blood (CB) in 4 (6%). Conditioning was MA in 57 (89%) and NMA in 7 cases (11%). Details of conditioning regimens were provided for 61 cases, in which 28 different combinations were used. Total-body irradiation (TBI) was used in 33 (54%) cases, with 2 others employing total lymphoid and thoracoabdominal irradiation. Overall, cyclophosphamide was used in 52 cases (85%): alongside TBI in 26 (44%) and busulfan in 16 (26%) cases. The 7 NMA transplants employed 6 different conditioning regimens. Overall, 90% received alkylating agents, although to infer this as an independent risk factor for DCL would be tautologous given the near-ubiquitous usage of alkylators in conditioning regimens. The heterogeneous nature of the few reported cases precludes identification of independent risk factors, with no significant trends in T cell depletion, engraftment kinetics, graft-versus-host disease (GVHD), cytomegalovirus (CMV) reactivation, or other viral complications emerging from the information available.

Standard leukemia relapse post-HSCT is considered a relatively early event. In a series of 6,691 patients disease free at 2 years, only 4.5% ultimately died of relapsed disease [58], whereas another revealed no relapses after 3 years [59]. In contrast, DCL appears to be a relatively later complication, with median time-to-DCL of 31 months (range: 2-312 months). (Where an antecedent MDS phase was diagnosed before evolution to frank leukemia, first diagnosis of MDS was considered as time of malignant transformation.) Time-to-DCL was significantly longer in those cases involving antecedent MDS, with or without overt transformation (median 43.5 versus 18.0 months; $P = .014$), again supporting the notion of distinct disease processes.

Methods Used To Confirm Donor Origin

Diagnosis of DCL depends on accurate and unequivocal demonstration of donor derivation of the leukemic clone. Until recent years this was difficult and hampered by many pitfalls. Several strategies have been employed, sharing the fundamental goal of identifying differences in genetic material between donor and recipient amenable to reliable investigation in the relapse clone. In practice, most approaches provide only indirect evidence of donor derivation through demonstration of complete donor chimerism (ie, lack of residual host hematopoiesis).

Conventional cytogenetics and fluorescent in situ hybridization (FISH)

Suspicion of donor origin in most early cases derived solely from the unexpected presence (or absence) of the Y chromosome in relapse BM specimens, in the context of sex-mismatched HSCT. Conventional banding techniques, developed in the 1960s, permitted differential staining of chromosomes cultured in metaphase and their identification based on distinctive banding patterns. Fialkow and coworkers' original DCL report concluded donor origin on the observation of Y chromosomes in all of 58 marrow metaphases on G-banding cytogenetics in a female transplant recipient [4]. The logical assumption was that leukemic transformation had occurred in engrafted donor cells, although the otherwise normal karyotype and lack of cell sorting precluded unequivocal confirmation that the visualized karyotype belonged to the leukemic clone. Nevertheless, at relapse, lymphoblasts represented 80% of total nucleated BM cells, so the finding of 46,XY in all metaphases inspected was considered sufficiently conclusive. Until the 1990s, demonstration of sex mismatch in this manner remained the mainstay of DCL diagnosis, occasionally supported by fluorescent Y body detection in interphase cells (a technique exploiting the bright staining of Y chromosome long arms with certain dyes).

However, conventional cytogenetics relies on capturing cells in metaphase and consequently requires active, spontaneous cell division. It is well recognized that leukemic cells can display lower proliferative rates than healthy cells (with clinical disease resulting from their gradual accumulation over time) [60]. Thus, a slowly dividing residual host leukemia could be outpaced by normal donor cells in culture and remain undetected, even when blasts vastly outnumber normal donor cells in BM. Certain methods used to prepare and capture metaphases can even suppress and select against leukemic cells in culture [61]. In contrast to healthy cells, leukemic metaphases are often of poor quality, because of inherent genomic instability and prior exposure to damaging chemo-/radiotherapy. Several early DCL reports noted a significant proportion of damaged, uninterpreble mitoses at relapse (eg, ~30% in case #3 [10]); potentially, these could have harbored an aberrant residual host leukemic clone. In case #1, whereas uncultured BM yielded only healthy looking 46,XY (donor) metaphases, most phytohemagglutin-stimulated cells demonstrated severe radiation damage with extensive chromosome fragmentation; only 4 were suitable for analysis, all of which displayed XX (host) karyotype and so conceivably might have represented the leukemic clone [4].

The introduction of FISH provided an additional tool for investigating origin of leukemic relapse, with the notable advantage of assessing cells in interphase, independent of mitotic rates. Despite yielding limited karyotypic information, FISH permits rapid enumeration of preselected chromosomes and DNA sequences to aid characterization of relapse clones and investigate donor-recipient chimerism. The ability of FISH to screen large numbers of cells (often $\geq$500) with fluorophores for sex chromosomes permits reliable detection of residual recipient hematopoiesis with sensitivity <1% [62]. The absence of mixed chimerism by FISH provided corroboratory evidence of DCL in at least 18 of the 28 cases of sex-mismatched DCL, after Mouratidou et al. first employed this approach in 1993 [24]. In some cases, FISH directly correlated the presence of new leukemia-defining autosomal abnormalities alongside donor-type sex chromosomes within the same cell (eg, Y and –7 in case #64), whereas in case #17, Lowsky et al. used FISH to localize –7 and XX (donor-karyotype) to myeloblasts morphologically [26]. Others inferred DCL from absence of minimal residual disease by FISH analysis in cases with a previously known cytogenetic marker.

However, any approach based on assessing chimerism by sex chromosomes remains applicable in only ~50% of transplant pairs, and will retain other limitations. Genomic instability and clonal evolution of leukemic cells frequently introduce new chromosomal abnormalities, and the propensity of blasts to lose or gain sex chromosomes is well recognized [63]. Y loss (–Y) is particularly common, observed in 3.4% to 6.3% of male leukemia patients [64,65] and even in healthy males with ageing [66]. Any altered X/Y-ploidy could potentially mislead toward erroneous suspicion of DCL. Anastasi et al. reported an ALL relapse in a male patient transplanted from his sister, involving 98% BM infiltration with normal female karyotype in 40 metaphases. However, the original disease clone had displayed complex karyotype, including gain of 2X chromosomes (XXXY). FISH of 1000 interphase nuclei revealed trisomy X (+X) in 83%, Y in 85%, and +17 (also present in the original clone) in 75%, confirming host origin of the disease clone [67]. Similarly, Perla et al. [68] disproved suspected DCL in a female CML patient displaying 46,XY karyotype at relapse, because FISH showed 2 times hybridization

signals in 78% of 500 interphase nuclei (making return of the original disease a more likely diagnosis) [68]. Although in these examples FISH correctly refuted DCL, this might not always have been the case. In DCL case #4, 42 of 64 relapse metaphases in a female recipient displayed 45,X0,t(8;21), with the absence of Y by FISH apparently confirming DCL [11]. However, the same picture could have resulted from relapse of the original disease with clonal evolution of new abnormalities: –Y/t(8;21); indeed, an earlier chloroma relapse had contained recipient-type XY cells only.

Molecular DNA markers

Some early DCL reports noted autosomal "markers" displaying a different staining intensity between donor and recipient on metaphase spreads. These were presumed to represent distinctive genetic polymorphisms capable of distinguishing their origin irrespective of sex. In case #3, the relapse 45,X0 karyotype left the donor (female) or host (male) derivation uncertain, but quinacrine staining revealed bright centromere fluorescence on chromosome 3 in donor fibroblasts that was lacking from pretransplant host BM cells [10]. A similar bright satellite on chromosome 21 in case #5 permitted diagnosis of DCL without sex mismatch for the first time [12]. Although the putative markers were not further characterized, these heralded future strategies of distinguishing donor from host based on idiosyncratic sex-independent genetic polymorphisms.

Subsequently, a variety of neutral DNA sequences (lacking known transcription function) were found to display considerable polymorphism within the population. Stably inherited as codominant Mendelian traits and propagated to all cells, these constituted a reliable set of markers for identifying cells originating from a particular individual and proved ideal targets for chimerism monitoring. Any transplant recipient may have up to 4 different alleles of a particular marker, depending on degree of zygosity, allele sharing, and chimerism between donor and recipient. Any *informative* donor allele (ie, absent in the recipient) can act as a useful marker to identify presence of donor-type hematopoiesis within the recipient. Restriction fragment length polymorphisms (RFLP) arise from variations in the length of DNA fragments generated after digestion by restriction endonucleases at specific cleavage sites. Variation results either from mutations altering integrity of enzyme recognition sites or from addition of neutral DNA between them (which can vary widely in size between individuals) [69]. The RFLP locus *D14S1*, identified in 1980 and localized to a highly polymorphic immunoglobulin heavy chain (IgH) region on chromosome 14, enabled Witherspoon et al. (case #8) to diagnose DCL following sex-matched HSCT [16]. Hybridization of DNA from recipient fibroblasts with a plasmid probe homologous to *D14S1* yielded bands of 19 and 16 kb, compared with bands of 16 and 12 kb in both relapse BM and donor mononuclear cells, thereby distinguishing the genotypes and confirming donor origin that would have remained unsuspected by standard methods. Feig et al. (#11) [20] and Orciuolo et al. (#32) [41] employed similar techniques; conversely, RFLP enabled Minden et al. to disprove suspected donor origin of AML in a male patient following BMT from his brother [70].

Some RFLPs were found to arise from polymorphic variations in number of tandemly repeated sequences of "junk" DNA in the nonencoding regions of genes. This heralded identification of *variable number of tandem repeats* (VNTRs), sequences 7-100 nucleotides in length generally concentrated at telomeres, and *short tandem repeats* (STRs): shorter (2-6 nucleotides), more numerous and widely dispersed throughout the genome. Both provide markers displaying high degrees of polymorphism, amenable to amplification by polymerase chain reaction (PCR) and capable of detecting persistence of a minor recipient-derived clone to high sensitivity [71]. STR-based techniques have largely superceded VNTRs owing to availability of commercial multiplex kits, ease of automation, and the high degree of STR polymorphism [72]. Tandem repeats were first applied to DCL in 1991 (case #12), in which PCR of 7 informative microsatellite sequences confirmed full donor chimerism in a male patient diagnosed with AML 9 months after sex-mismatched BMT—supporting the observation of 46,XX,t(9;11) in 20/20 metaphases. The stated sensitivity of the test ("0.01%") effectively excluded the possibility of a recipient clone of sufficient size to explain the observed 90% BM infiltration by AML [21].

Since 1993, at least 39 (of 51) DCL diagnoses relied predominantly on 100% donor chimerism by VNTR or STR PCR. However, these approaches too carry limitations. Although applicable equally to sex-matched and -mismatched transplants, at any locus, 25% of sibling pairs will have inherited identical alleles and thus lack an informative marker. Moreover, in practice, coamplification of alleles generally limits the detection sensitivity of current VNTR/STR PCR techniques to ~1% of the minor component (ie, 1 recipient cell in ~100). With substantial allele size difference, preferential amplification of smaller alleles may cause allelic dropout and further reduce detection sensitivity of the larger allele. PCR-based assays are prone to contamination, with proportionally greater impact at higher levels of sensitivity. Theoretically, these permit low-level residual recipient hematopoiesis to persist undetected, although by definition, DCL must involve a proportion of leukemic blasts well in excess of the detection limits of the test.

Importantly, total/partial chromosomal deletions in the inherently unstable disease clone will remove

any marker/s located on the corresponding segment, with loss of heterozygosity and potentially misleading results even for a theoretically informative marker. For example, where a single allele is shared by both parties, loss of the unique informative donor allele from the disease clone would convert the apparent genotype to that of the recipient. Stein et al. highlighted this potential source of error in a male patient following BMT from his sister [73]. Cytogenetics initially suggested DCL, with 46,XX karyotype, negative Y-body fluorescence, donor-type banding heteromorphism on chromosome 9qh, and absence of the original t(18;21) from all visualized metaphases. Two RFLP probes (*pDP105*, *pDP132*) initially corroborated suspicion of DCL. However, these loci are situated on the Y chromosome, and RFLPs from chromosomes X (*St14-1/S-232*), 5p (*L4-123*), and 21q (*L4-427*) revealed >90% recipient chimerism, confirming hematopoiesis to be dominated by recipient-derived lymphoblasts. Schichman et al. described 2 instances of informative STR allele loss because of hypoploidy in 140 HSCT recipients [74]. One involved a female CML patient relapsed in blast crisis after sex-mismatched BMT, with 46,XX,t(9;22) and partial deletion of chromosome 3, consistent with evolution of the original clone. Both donor and recipient were heterozygous for the *D3S1358* STR (on chromosome 3p) with no shared alleles. Although host derivation of relapse was confirmed by presence of the recipient-type *18kb* peak, the authors noted absence of the other recipient allele (*16kb*), consistent with the single chromosome 3p deletion. Had the deleted *16kb* peak been the only informative allele (ie, donor homozygous for the *18kb* marker), DCL might have been erroneously suspected. Similarly, Zhou et al. [75] reported an initially aberrant result mimicking 100% donor engraftment from VNTR PCR of the *apoB* locus in a patient with near-haploid karyotype at CML relapse.

Therefore, several markers across different chromosomal loci should always be sought and correlated with evaluation of the relapse karyotype. However, without an unequivocal marker of clonality the apparent absence of recipient hematopoiesis remains only an indirect indicator of DCL. Spinelli et al. [76] reported presumed DCL in a male patient transplanted from his sister for Philadelphia-positive ALL, in which DCL was suggested by 46,XX karyotype, absence of the Y-specific *DYS14* VNTR and 100% donor-type *YNZ-22* VNTR (chromosome 17) by PCR. Nevertheless, the authors considered the possibility of comigration of 2 alleles, and sequencing of the CDRIII region of the *IgH* gene revealed a unique DNA rearrangement identical to that in the original disease clone, proving relapse to be recipient derived. The authors advocated need for "accurate and extensive molecular characterization to prove donor origin of a leukemia relapse" with identification of leukemia-specific sequences perhaps the only definitive tool for this purpose.

Nevertheless, provided a sufficient number of informative alleles are examined, STR chimerism analysis should reliably confirm DCL in most cases, and represents a major advance on earlier methods reliant solely on sex mismatch by conventional cytogenetics. Consequently, the validity of some early DCL diagnoses have been questioned, and it is noteworthy that in 8 of the first 10 reports, DCL type was identical to that of the original disease (true only in 10 of the subsequent 54 cases, most of which employed molecular techniques). Newer diallelic polymorphic markers including single nucleotide polymorphisms and short inversion/deletion polymorphisms have the additional advantage of permitting amplification of each allele separately, negating the effects of competitive amplification and permitting more sensitive detection of mixed chimerism [77,78]. Investigating origin of relapses in future should be enhanced by their potential for superior sensitivity (approaching 10^{-4} to 10^{-6} of the minority cell population [79]).

Etiology of DCL

The leukemic transformation of previously healthy donor HSCs provides a useful in vivo model for investigating the mechanisms involved in leukemogenesis. It presents a unique opportunity to prospectively study the events experienced by HSCs leading to their malignant transformation, because leukemia in the general population is usually sporadic and unpredictable, whereas HSCT patients are routinely subjected to relevant and repeated investigations; these are informative prospectively but also provide useful samples for retrospective analysis. From the limited data available, any discussion of the mechanisms driving donor cell transformation is highly speculative, and the heterogeneity of documented cases precludes identification of a single mechanism or unifying hypothesis. Nevertheless, some published cases provide intriguing clues, and several putative theories have been proposed (Figure 2). DCL oncogenesis is probably a multifactorial process involving the convergent interplay of different influences. A multiple "hit" hypothesis appears plausible, by which HSCs primed within the donor (eg, an inherited primary caretaker gene defect) experience second/subsequent critical "hits" after transplantation into an aberrant donor environment, unusually supportive for realization of this malignant potential. A high frequency of antecedent MDS and complex karyotypes perhaps supports the notion that multiple genetic events might occur in at least some DCL cases.

Occult leukemia in the donor

The simplest explanation for apparent DCL would be that of an undetected malignant clone present

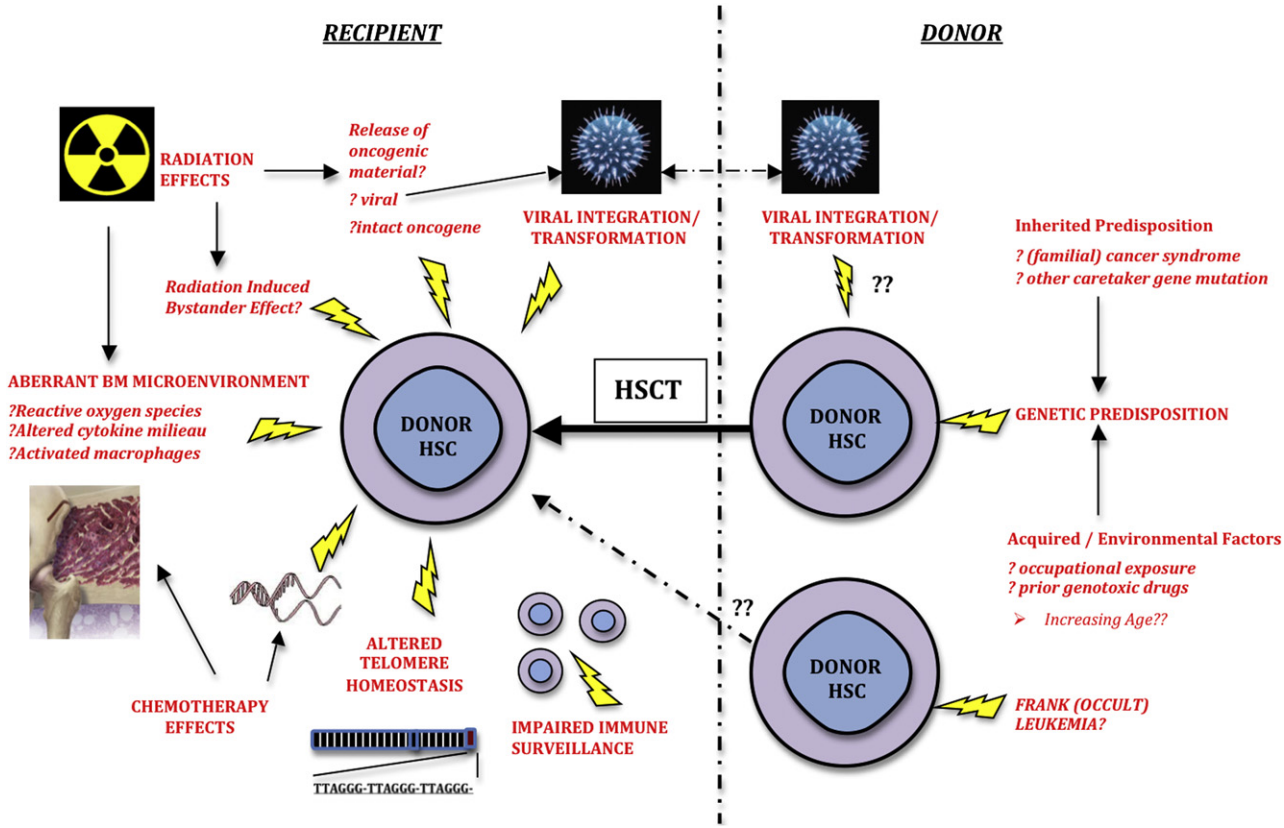

Figure 2. Proposed mechanisms and other etiologic factors influencing development of donor cell leukemia (DCL). Several putative mechanisms have been proposed to contribute critical "hits" toward leukemogenesis in cells of donor origin following transplantation into a new host. Genetic factors might prime stem cells with a preleukemic phenotype within the donor, with a range of recipient- and therapy-specific factors probably interacting to contribute toward realization of malignant potential following engraftment into the more conducive bone marrow environment of the recipient. A "multiple hit" hypothesis has been proposed, with DCL probably the convergent endpoint of numerous distinct pathways (which may vary between individual cases).

within the donor at time of donation. Indeed, Niederwieser et al. [80] have reported inadvertent transmission by BMT of occult AML into a CML patient, in which the donor had been aleukemic at donation but developed frank AML shortly afterward. Six months after infusion of the contaminated graft, AML became clinically evident in the recipient, displaying identical morphologic, phenotypic, and cytogenetic characteristics to the donor's disease [80]. Accidental transplantation of CML [81] and cutaneous T cell lymphoma [82] have also been reported. Transfer of even a small minority clone can result in clinical disease. Mielcarek et al. described long-term engraftment and clonal dominance of an MDS clone transplanted from an asymptomatic 50-year-old female into her sister [83]. The donor's MDS was diagnosed retrospectively, characterized by del(20q) in 18% BM cells. Cells bearing del(20q) steadily increased in the recipient, from 10% after 5 months to 73% at 7 years. Because T and B cells were part of the abnormal, clone del(20q) must have arisen in a multipotent progenitor cell, demonstrating capacity of a low-level donated clone to ultimately dominate hematopoietic reconstitution.

Sala-Torra and coworkers' [7] series included 6 examples of hematologic malignancy directly transmitted through HSCT (3 CLL; 1 each marginal zone lymphoma, mantle cell lymphoma, ALL). Disease clones detected in recipients displayed identical immunophenotype and/or clonal *IgH* gene rearrangement to the corresponding donor's disease. Inadvertent transmission of malignant clones might be surprisingly common, and intuitively might increase with the rising popularity of NMA HSCT and corresponding increase in age of sibling donors. Niederwieser et al. [84] addressed this in detail and outlined stringent recommendations for donor workup. Kiss et al. [85] starkly demonstrated the potential risk, having discovered 3 incidental malignancies (myeloma, CLL, MDS) in potential donors during routine workup BMs in a single year. However, no donors were diagnosed with leukemia in the 46 cases of *idiopathic* DCL for which donor follow-up was reported (median follow-up 48 months). Many had been recalled for PB/BM screening, and extended literature search revealed no updated reports of leukemia in any of the donors in question.

Preleukemic potential in donor cells

Alternatively, outwardly healthy donor HSCs might have been previously endowed with genetic premalignant potential (either inherited or acquired). Hereditary predisposition to MDS/AML has been described with *CEBPA* and *RUNX1* gene mutations, and 12 pedigrees with familial –7 and MDS/AML have been identified [86]. A high incidence of MDS/AML also occurs in several familial cancer predisposition syndromes, characterized by germline mutations in tumor suppressor/DNA repair genes, genomic instability, and accrual of oncogenic mutations. Li Fraumeni syndrome (associated with *p53* defects) carries an estimated leukemia risk of ~10% [87], whereas the chromosomal breakage disorders Bloom's syndrome and Fanconi anemia confer lifetime MDS/AML risk of 20% to 25% and 35% to 50%, respectively [88,89]. Even without a defined syndrome, Shpilberg et al. [90] reported an odds ratio of 3.62 for hematologic malignancy in relatives of 189 affected patients; most diseases differed from the index case, suggesting a defect in a pluripotent HSC with additional environmental factors dictating progression to different lineage-specific malignancies.

In the 80% of DCL cases involving related donors, any genetic predisposition might have been shared by both parties, with malignant potential more likely to be realized in the favorable posttransplant microenvironment of the recipient. In case #32, both recipient and donor developed MDS within weeks of each other 18 years after HSCT. STR analysis confirmed donor-derivation of the recipient's disease, yet the 2 sisters had led independent lives, in different areas with unrelated lifestyles. The authors concluded the late contemporaneous onset of similar disease to suggest intrinsic damage in the donor HSCs themselves [41]. Interestingly, although the recipient's disease was rapidly progressive, the donor displayed only modest cytopenias and stable blast percentage, suggesting a degree of immunologic control in the immunocompetent donor. The donor in case #28 developed high-grade non-Hodgkin lymphoma (NHL) at age 17, 13 years after donating BM to her brother. A mutation at position 2281 of the *BLM* gene was later detected in the donor, consistent with Bloom's syndrome; the recipient had died (of DCL) and could not be tested [37]. In case #25, the donor (a lifelong nonsmoker) developed squamous cell bronchial carcinoma 15 months after donating BM to his brother, who developed DCL 33 months later. Screening for a *p53* mutation was negative, but 3 unrelated malignancies in 2 siblings strongly suggested a shared genetic predisposition to malignancy [34]. The donor in case #35 developed breast cancer 10 months after donating [44]. Although familial predispostion was not mentioned in other cases, detailed pedigree analysis was not presented, and it is unlikely that donors had been routinely screened for such mutations. Moreover, in Li Fraumeni syndrome, only ~50% to 70% patients have an identifiable *p53* mutation, and other syndromes display variable penetrance. Apart from the few well-described syndromes, much about familial predisposition to cancer remains obscure.

With an unrelated donor, the likelihood of shared predisposition becomes much less plausible, and only 9 cases of DCL have been reported in this setting. Nevertheless, VUD HSCs might still harbor premalignant potential, due either to coincidental genetic predisposition or environmental exposure to genotoxic stimuli. The donor in case #17 had previously received the alkylating agent dacarbazine for malignant melanoma, which might have rendered donor HSCs sensitive to secondary stresses following transplantation; the finding of –7 was consistent with a therapy-related AML. The donor remained healthy after 9 years [26]. Although no specific donor risk factors were described in any other cases, relevant background information (eg, drug/employment history) was rarely presented.

An interesting observation is the frequency with which latent premalignant clones occur in the normal population. *Bcr-abl* mRNA was found in ~30% of normal adults [91] and t(14;18), a recurring abnormality in follicular lymphoma, was detectable in 24% of healthy individuals [92]. Latent abnormal clones might be particularly common in CB donations. Uckun et al. [93] showed that *MLL-AF4* fusion transcripts characteristic of infant ALL were present in ~25% of fetal BM samples. PCR screening of CB samples revealed a significant frequency of potentially preleukemic clones (~1% *TEL-AML1*, ~0.2% *AML-ETO*) [94], but the ~100-fold lower incidence of subsequent leukemia indicates a need for additional postnatal events. Up to 5% of CB samples might harbor potentially preleukemic clones, raising the possibility that DCL might be disproportionately common after cord blood transplantation (CBT) [95].

Viral transfection/integration

The notion of a leukemogenic virus has always been attractive, not least for DCL, in which recurrence of a similar (but distinct) disease develops within an individual despite eradication of the original culpable clone. Parallels have been drawn with other neoplasms of proven viral etiology. The neoplastic lymphoid proliferation of posttransplant lymphoproliferative disorder (PTLD) is driven by EBV infection of B-lymphocytes, which in the context of dysregulated T cell immunity can facilitate B cell immortalization and uncontrolled proliferation [96]. Although PTLD following solid organ transplantation is usually recipient derived HSCT-associated PTLD is almost always donor in origin. This holds even when the donor is

EBV-seronegative, suggesting that viral transfection into donor cells occurs after transplantation. However, PTLD is driven at the level of the mature lymphocyte, and it is difficult to purport an analogous role for EBV in transforming the more primitive cells responsible for DCL. ATL is another aggressive lymphoproliferative disorder, occurring exclusively in the context of infection by the retrovirus HTLV-1. Although mechanisms are not fully understood, roles for the HTLV-1–encoded proteins Tax (which can inactivate *p53* and *p16*) and HBZ (which supports T cell proliferation) appear likely. However, only 2% to 6% of HTLV-1 carriers develop ATL (after a latency of ~50-60 years from infection), implicating roles for additional genetic and epigenetic factors [97]. Examples of donor-derived ATL following HSCT have been described, with uniformly shorter latency than for standard ATL. Tamaki and Matsuoka reported a recurrence of ATL (in PB) 4 months after HSCT for lymphoma-type ATL. Source of infecting virus was uncertain, because both recipient and donor were HTLV-seropositive, but STRs confirmed 100% donor engraftment [98]. Ljungman et al. detected HTLV-1 in PB lymphocytes of an ATL patient following HSCT from an HTLV-1–seronegative sibling, demonstrating the capacity for leukemogenic viral material within the host to rapidly transfect virus-naïve donor cells [99]. Although neither PTLD nor ATL are directly analogous to DCL, the potential for prior therapy to release a comparable, hitherto-unidentified oncogenic virus for transfection into donor HSCs has been proposed (although supportive evidence is lacking).

Oncogene integration/fusion

Some have taken this idea further and speculated that other (nonviral) leukemogenic material, most likely a dominant oncogene, might be released by conditioning from residual leukemia cells and directly transfect the genome of donor HSCs. Theoretically, the radiation damage rendered by TBI could release intact portions of DNA, which might survive in a viable state to be incorporated into donor HSCs proliferating nearby. However, even allowing for a degree of clonal evolution, the ensuing DCL phenotype should closely resemble that of the original disease; this was only observed in 18 cases (28%). Alternatively, leukemic cells surviving conditioning might fuse with incoming donor HSCs, with diploidization yielding cell lines bearing recipient-derived leukemic factors and donor-derived chromosomes. The notion of cell fusion as a mechanism of oncogenesis dates from the 19th century when Hansemann proposed malignancy to result from hybridization between leucocytes and somatic cells. Whereas somatic cell fusion usually results in terminally differentiated multinuclear cells incapable of proliferation, malignant cells display high rates of spontaneous fusogenicity, resulting in proliferating hybrids characterized by a high degree of aneuploidy [100]. Mechanisms remain unclear, but certain viruses can mediate cell fusion via specific proteins [101], and Duelli et al. [102] showed that an otherwise harmless virus caused massive chromosomal instability through fusing cells whose cycles had been disrupted by oncogenes, with some producing aggressive, aneuploid epithelial cancers in mice. Whether these have relevance to DCL is unclear, but the opportunity for such fusions would theoretically exist in the posttransplant environment, and could conceivably cause residual host disease clones to masquerade as DCL at relapse. Notably, many cases of DCL displayed complex (often unusual) karyotypes, with aneuploidy in 30%.

Residual effects of cytotoxic chemotherapy

HSCT recipients are at significantly increased risk of secondary malignancies. One study of 557 BMT recipients revealed a cancer rate 5.13 times higher than expected, with actuarial risk 4.2% at 10 years. Most were epithelial carcinomas of the skin and mouth (45.7%), but hematologic malignancies accounted for 11.4% [103]. Although multifactorial, the carcinogenic effects of cytotoxic chemotherapy play a major role. Therapy-related AML (t-AML) comprises 5% to 10% of all AML cases, having been appreciated first in long-term survivors of Hodgkin lymphoma [104]. Two distinct patterns have been described. The more common occurs 5 to 7 years after exposure to alkylating agents, often preceded by an antecedant MDS phase (typically lasting 6-12 months) and characterized by deletions involving chromosomes 5 or 7, +8, +21, unbalanced translocations, and complex karyotype. The other occurs 1 to 5 years after exposure to topoisomerase II (TII)-inhibitors. TII inhibition prevents efficient unwinding of DNA during replication leading to DNA strand breaks; intranuclear religation can facilitate illegitimate, nonhomologous recombinations, forming potentially leukemogenic translocations commonly involving 11q23 and 21q22 (sites of *MLL* and *AML1* genes, respectively). Other associations include deletions of 20q and 12p [105]. *MLL*-rearranged t-ALL has also been reported [106].

Most HSCT patients have been exposed to these agents, with 96% having received either an alkylator or TII-inhibitor with conditioning. Another (#35) had received busulfan with an earlier CBT [44]. Although timing of HSC infusion is designed to prevent direct exposure to genotoxins, effects might conceivably persist to influence incoming donor HSCs, either directly or via effects on stromal elements. Commensurate with this is the frequent observation of characteristic cytogenetic abnormalities in DCL. The malignant clone displayed –7 in at least 10 cases, either alone or with other abnormalities. Two others displayed deletions/translocations involving chromosome 7, whereas *MLL* or *AML1* gene rearrangements

occurred in 5 cases each. In total, cytogenetic abnormalities typical of t-AML occurred in at least 47% of DCL cases. Moreover, in some cases, engrafted donor HSCs were further exposed to chemotherapy in vivo within their new host. For example, patient #62 had received etoposide and tenoposide for an earlier recipient-derived ALL recurrence post-HSCT; the subsequent DCL displayed *MLL* rearrangement [56]. Stevens described DCL arising in cells from a first donor after successful HSCT from a second (case #61). Both transplants were conditioned with fludarabine and cyclophosphamide, exposing the original donor's stably engrafted HSCs to potentially genotoxic chemotherapy; MDS/AML with –7 developed in cells of first donor origin 10 months later [55].

Bystander radiation damage

Therapeutic irradiation with conditioning has been linked to DCL leukemogenesis via several mechanisms. Marmont et al. suggested that oncogenic material, such as a virus or intact oncogene, might be physically released from host leukemic cells by radiation damage and transfect into DNA of incoming donor HSCs [13]. Havelange et al. [51] hypothesized that radiation might have an enhancing effect on certain oncogenic viruses. Leukemia is certainly among the most radiogenic of all neoplasms, with a relatively short latent period from exposure [107,108]. Studies into the specific leukemogenicity of therapeutic irradiation have often been confounded by concomitant cytotoxic chemotherapy; nevertheless, both pretransplant radiotherapy [109] and TBI conditioning [110] have been identified as independent risk factors for secondary MDS/AML after autologous HSCT.

Established dogma in radiation biology attributed carcinogenicity to direct energy deposition in the nucleus, with DNA ionization converted to potentially leukemogenic mutations during replication or enzymatic DNA repair. Intuitively, the timing of HSC reinfusion should protect against these direct effects. However, this paradigm has been challenged by recognition of a *radiation-induced bystander effect* (RIBE), by which equivalent biologic effects are observed in nonirradiated cells, either located nearby or exposed to medium from other irradiated cells. Studies in the 1950s observed changes in sternal BM of children receiving splenicir radiation for CML [111], whereas later findings that plasma from irradiated patients could induce chromosomal damage in nonirradiated cells in culture [112] suggested the generation of "clastogenic factors": soluble damage signals released into plasma on exposure to ionizing radiation capable of inducing bystander DNA damage to distant cells in vivo or nonirradiated cells ex vivo. Clastogenic signals retain functional ability in circulation as demonstrated by their ability to cross the placenta and induce chromosomal instability in children whose mothers received nonpelvic irradiation during pregnancy [113]. They also persist in vivo for long periods. Goh [114] observed increased chromosomal breakages in leukocytes from normal individuals when cultured with serum from patients irradiated 10.5 years previously, whereas transferrable clastogenic activity has been observed in plasma of atomic bomb survivors [115] and Chernobyl workers [116] even decades after exposure. Studies at the cellular level have demonstrated multiple endpoints of biological damage in bystander cells, including micronucleus formation, *p53* up-regulation, genomic instability, mutagenesis, and frank malignant transformation in cell lines [117].

The identity of putative clastogenic factors and mechanisms by which they are perpetuated remain uncertain. Some evidence suggests dependence on gap junction (GJ)-mediated signaling, given that bystander mutations in cell lines could be inhibited by the GJ inhibitors lindane and octanol and in cells lacking GJ formation [118]. However, evidence from medium transfer experiments and the observation of distant bystander effects confirm that dependence on physical interaction is not universal. Several candidate soluble clastogens have been proposed, including aldehydic breakdown products of lipid peroxidation [119], inosine nucleotides [120], and various cytokines [121]. Oxidative stress and reactive oxygen species (ROS) appear important because bystander damage is inhibited by the ROS scavenger superoxide dismutase [122]. ROS can cause various DNA lesions including potentially leukemogenic mutations, and can activate signaling pathways (*p53*; *MAPK*) in bystander cells [123]. ROS might also induce a self-perpetuating cycle of oxidative stress (and persistence of clastogenicity) by stimulating competent cells to generate further ROS.

From the model of RIBE, it is tempting to speculate that TBI might render donated HSCs subject to similar bystander effects in vivo. Comparisons with the medium transfer experiments are particularly compelling, given that HSCT represents an analogous (albeit reversed) context, that is, transfer of *cells* into irradiated *medium*. In some situations, recipient cells might persist long enough to deliver damage signals through direct physical contact with incoming donor HSCs; alternatively, soluble clastogenic factors generated by irradiated host cells might persist and circulate to induce genotoxic effects on donor cells, contributing critical *hits* toward a leukemic phenotype. Perhaps the most compelling in vivo evidence derives from experiments in which mice with inherent plasmacytomata were administered melphalan to create a model of HSCs imbued with preleukemic potential; when splenocytes were transferred into preirradiated syngeneic mice, almost all developed acute leukemia of donor origin, implicating a direct leukemogenic effect of prior irradiation on unstable (but radiation-naïve) donor cells [124]. Although conditioning included

irradiation in only 57% of DCL cases, other recipients were exposed to earlier therapeutic (and possibly occupational) irradiation with potential for longstanding clastogenicity. The ALL patients in cases #10 and #11 had received cranial irradiation pretransplant [19,20], whereas patients #4, #7, #18, and #28 (3 conditioned with chemotherapy alone) received radiotherapy posttransplant for recipient-derived extramedullary relapses, 15 to 21 months before onset of DCL [11,15,27,37].

Defective marrow stroma/microenvironment

The BM stroma is a complex microenvironment of fibroblasts, adipocytes, osteoblasts, macrophages, and endothelial cells in a supportive matrix of insoluble proteins, within which crosstalk between stromal components and hematopoietic progenitors is mediated by numerous chemokines/cytokines. Complex interactions are essential for supporting healthy hematopoiesis, and perturbation of this equilibrium might play a role in leukemic transformation in vivo. Most evidence is indirect, but stromal cell layers from patients with MDS/AML frequently display abnormal cytokine milieau [125], and leukemia-associated chromosomal abnormalities have been identified in stromal cells of MDS and AML patients [126].

The BM stroma plays a key role in facilitating and supporting hematopoietic reconstitution following HSCT, as inferred from the observation of augmented engraftment after cotransplantation of HSCs with ex vivo–expanded mesenchymal stem cells [127]. Close physical interaction invariably occurs between donor HSCs and BM stromal cells, which probably remain of host origin after HSCT [128]. In the context of DCL, abnormalities of the recipient microenvironment might be an inherent feature (given its support in developing 2 distinct malignancies), or could result from damaging effects of previous treatment. Chemo-/radiotherapy can inflict considerable damage on BM stromal elements [129], whereas radiation-induced stromal damage can aid survival of HSCs in culture and contribute toward selection and proliferation of leukemic clones [130]. Radiation-induced DNA damage induces a stress response intended to prevent proliferation of damaged cells resembling an inflammatory-type response, including overproduction of ROS [131]. Indeed, subclinical markers of inflammation remain significantly elevated (in proportion to radiation dose) >50 years after exposure [132], and murine evidence has directly linked induction of inflammation with leukemogenesis after irradiation [133]. Fibroblasts and macrophages can permanently arrest in an activated state, continuously generating ROS, probably mediated by stromal overproduction of transforming growth factor (TGF)-β [134], which itself promotes survival and proliferation of AML cells in vitro [135]. Experiments have shown higher levels of ROS in HSCs cocultured with irradiated BM stromal cell lines [136], suggesting that stromal elements rather than irradiated HSCs might provide the major source of ROS responsible for RIBE. Inflammatory macrophages are a potent source of microenvironmental ROS, and although ultimately donor derived following HSCT they are relatively radio-/chemo-resistant and may take months to be fully replaced by differentiation of donor-derived monocytes [137]. Their role in carcinogenesis is increasingly recognized [138], and abundant interactions between host macrophages and donor HSCs likely occur in the immediate aftermath of HSCT.

The intensive chemical and radiation exposure experienced by the recipient BM microenvironment might trigger comparable inflammatory activation within the stroma, resulting in increased ROS production and elevated leukemogenic potential. Other alterations to stromal composition or function might alternatively contribute to DCL through novel mechanisms. In a recent review, Flynn and Kaufman [139] suggested that DCL leukemogenesis might not be cell autonomous proposing that dysregulation of the normal cellular signalling processes play an integral role. Such an approach represents a hallmark shift in emphasis away from the stem cell and onto the microenvironment in understanding the mechanisms of leukemogenesis.

Impaired immune surveillance

Ordinarily, tumor surveillance prevents autochthonous neoplasia through complex interactions involving all branches of a competent immune system, to recognize, eliminate, and prevent tolerization to spontaneously arising malignant clones. Tumor development is associated with acquisition of tumor-*specific* antigens, providing targets for recognition by host cytotoxic T-lymphocytes. Progression to clinical malignancy usually requires induction of *anergy* to the malignant clone [140]. Alternatively, the magnitude of an immune response may be insufficient to quell a rapidly expanding and mutating tumor, because T cells recognizing any particular antigen are usually present in low numbers even in a normal T cell repertoire. T-immunodeficiency, an obligate phenomenon following allogeneic HSCT, predisposes to this outcome.

T cells remain quantitatively and qualitatively suppressed for long periods after HSCT. T-reconstitution initially involves expansion of a donor-derived postthymic T cell repertoire transferred with the graft, primarily memory T cells. These have already undergone thymic selection in the donor and so display tolerance to donor-derived cells. Exposure to reactivating viruses, minor HLA antigens, and antigens expressed by residual leukemia cells result in massive but uneven T cell expansion, with a skewed repertoire of TCR affinities [141]. Full immune recovery involves emergence of a new T cell repertoire generated from

donor-derived naïve (prethymic) T cells, subjected to thymic maturation and selection, during which self-tolerance is achieved. Ideally, this results in a fully competent mature T cell repertoire, with tolerance against both recipient cells and donor-derived hematopoietic cells. However, T-reconstitution is highly variable and influenced by several factors. The number of donor T cells present in the graft varies, with significantly fewer in BM and CB than PB; furthermore, T-depletion was deliberately employed in at least 14 DCL cases. Even when T-replete, the early expansion challenge faced by the graft on exposure to a massive new antigenic environment could overwhelm the relatively few competent donor T cells, resulting in a period of suboptimal surveillance during which responses to transformed donor HSCs might be impaired. Furthermore, the ubiquitous pharmacologic immunosuppression following HSCT has profound suppressive effects on T cell function, whereas chronic GVHD (cGVHD) itself is immunosuppressive and may predispose to secondary malignancies [142]. Details of posttransplant course were not consistently or extensively presented in DCL reports, but 23 cases confirmed that significant cGVHD had occurred. Regeneration of a mature T cell repertoire also relies on adequate thymic function in the recipient. This decreases markedly with age, and studies have shown distinct age differences in the capacity of the host thymus to *educate* donor-derived prethymic precursors [143]. Thymic function may be additionally damaged by prior chemo-/radiotherapy. Consequently, abnormalities in T cell maturation and function can persist, perhaps indefinitely, after HSCT [144]. Impaired function of antigen-presenting cells (APCs) might also contribute, with dendritic cells known to display persisting functional abnormalities in the posttransplant period [145].

A pertinent observation is the transient appearance (and spontaneous disappearance) of potentially preleukemic clones in the early posttransplant period. In Palka and coworkers' study of 31 BMT recipients, 1 developed an abnormal clone (45,XX,–16) at 30 months, which later disappeared spontaneously [18]. Sevilla et al. reported a transient donor-derived MDS (with –7) in a 4-year-old girl after CBT [146]. At 3 months, she displayed frank pancytopenia with morphological features of MDS in BM and –7 in 79% of nuclei by FISH; however, by 12 months, her blood count had spontaneously returned to normal, and at 2 years, –7 was present in only 5.5% of cells. The authors concluded that early clonal instability and impaired immune surveillance had facilitated engraftment by a clone bearing –7, but that immune reconstitution enabled its gradual eradication and substitution by progeny of normal HSCs responsible for long-term engraftment [146]. Transient –7 with spontaneous remission has also been described in normal children, in whom postthymic T cell maturation is an active and dynamic natural process [147]. T cells present in CB grafts are naïve, because the fetus is exposed to few alloantigens in utero; as more CBT are performed, it will be interesting to observe the prevalence and fate of abnormal clones, and whether the paucity of postthymic passenger T cells translates into increased risk of malignancy.

Telomere shortening and replicative stress

Telomeres are nonencoding regions of DNA flanking the ends of chromosomes, comprising a variable number of tandemly repeated TTAGGG motifs and associated proteins. They play a critical role in the maintenance of chromosomal integrity. Because DNA replication by DNA polymerase is a unidirectional process, loss of a small segment from the 5′ end of each strand inevitably occurs with each cell division. This "end-replication problem" results in progressive telomere shortening (by ~50 to 100 bp per division [148]). In postmitotic cells, critical telomere shortening usually results in *senescence*, a state of irreversible cell cycle arrest triggered by recognition of the unprotected end as a conventional DNA break capable of inducing *p53*-mediated DNA repair. Consequently, most somatic cells have a finite lifespan, becoming senescent after 40 to 80 population doublings in vitro [149]. Certain cells, however, maintain telomere length and proliferative capacity through expression of telomerase, a reverse transcriptase capable of synthesizing terminal nucleotides. Telomerase expression is variably high in cells endowed with self-renewal capacity, including HSCs [150], and correlates strongly with histologic evidence of malignant cells in many tissues [151]. Although critical telomere shortening usually triggers senescence, cells with dysfunctional *p53* or other DNA repair pathways (and lacking telomerase) continue to proliferate until entering *crisis*, a state characterized by extremely short telomeres, genomic instability, and widespread apoptosis. Unprotected telomere ends of proliferating cells in crisis are prone to illegitimate fusion and generation of complex, nonreciprocal, potentially carcinogenic translocations [151].

Although defective telomere homeostasis is implicated in many tumors, studies in hematologic malignancies are confounded by the variable telomerase expression of normal HSCs. Nevertheless, progressive telomere shortening is described in leukemia, and although this might be a result (rather than cause) of rapid clonal expansion, critical telomere shortening and resultant genomic instability might promote disease evolution. Shorter telomeres and telomerase up-regulation have been observed in AML blasts at diagnosis and relapse (compared with remission samples [152] and healthy controls [153]). Higher telomerase levels have also been correlated with presence of

complex cytogenetic abnormalities [154] and poorer prognosis [155]. Swiggers et al. showed universally short telomeres in AML blasts displaying chromosomal aberrations that could theoretically result from telomere dysfunction, suggesting that telomere shortening may contribute to chromosomal instability in AML development [156]. Patients with dyskeratosis congenita, an inherited BM failure syndrome characterized by defective telomere maintenance, also display significantly higher rates of MDS/AML [157].

Under normal circumstances, adult HSCs generate $\sim 10^{11}$ blood cells each day. Thus, even homeostasis represents a considerable replicative challenge, but rapid induction of senescence is largely abrogated by high telomerase expression. Despite containing relatively few HSCs, the donor graft faces a still greater proliferative demand to rapidly repopulate the recipient's entire hematopoietic system. The mitotic rate required to meet this expansion challenge imposes considerable replicative stress and corresponding increase in telomere attrition. Several studies have documented significant (and accelerated) telomere shortening following HSCT. Notaro et al. [158] observed consistently shorter telomeres (by 79 to 1,446 bp) in granulocytes of 11 HSCT recipients compared with respective donors, inversely correlating with nucleated cell dose infused. Wynn et al. [159] detected shorter telomeres (by mean 400 bp) in leukocytes of 14 HSCT recipients compared with donors, equivalent to an estimated 15 years' aging; others have estimated telomere attrition equivalent to >50 years' aging in normal controls [160]. Similar trends were observed after NMA HSCT [161], implicating replicative stress (rather than effects of conditioning) as the principal mediating factor. Interestingly, this appears restricted to the first year posttransplant, with only minor telomere shortening (comparable with normal levels) thereafter [162]: kinetics that intuitively fit with a period of early replicative stress followed by normalization once recipient hematopoiesis is restored. The combination of replicative stress, altered telomere maintenance, and genomic instability might predict the early onset of clonal hematopoietic disorders in engrafted donor cells.

Treatment of DCL

DCL is considered to carry an extremely poor prognosis, consistent with other secondary leukemias. Overall, this appears to hold, with at least 34 patients (53%) having died before publication, at a median 5.5 months after DCL diagnosis (range: 1 week-64 months). However, at least 24 of the remaining 30 were evidently alive at publication, after median follow-up of 14 months. Prognostication is hampered by the small number of reported cases, variable follow-up, and range of treatment strategies employed. Nevertheless, durable responses to salvage treatment were clearly achieved. Of the 24 surviving patients, at least 17 (71%) were reportedly in complete remission (CR) at time of publication, with 4 others on active treatment. Mean overall survival (OS) for treated patients was 32.8 months (95% confidence interval [CI]: 22.5-43.1) (Figure 3).

Reinduction chemotherapy with curative intent was attempted in 47 (of 52) DCL patients, employing a range of standard AML- or ALL-specific regimens (Table S1). No/minimal response occurred in 16 cases, and in another, reinduction resulted in aplasia precluding further treatment (case #43) [6]. Nevertheless, CR was achieved in at least 27 patients, remaining durable in 16 of these. CR was achieved in 13 of 17 (76%) donor-ALL patients, with 7 still in remission at median 12 months follow-up. At least 16 of 34 (53%) donor-AML patients entered CR, with another still on treatment; remissions persisted in at least 10 of these (33%) at median 17 months follow-up. A second allogeneic HSCT was performed in 17 patients of whom at least 7 (41%) remained alive (in CR) at a median of 29 months from diagnosis of DCL. Three patients were awaiting second HSCT (in CR) at time of publication. At least 7 patients subjected to second HSCT subsequently relapsed and died. One patient achieved a 44-month remission with reinduction before relapse of DCL prompted second BMT, but despite again responding, she relapsed and died 12 months later. Salla-Torra and coworker's cohort included 3 DCL cases whose treatment included second HSCT, and although patient fate was not clearly stated, durable responses were probably achieved

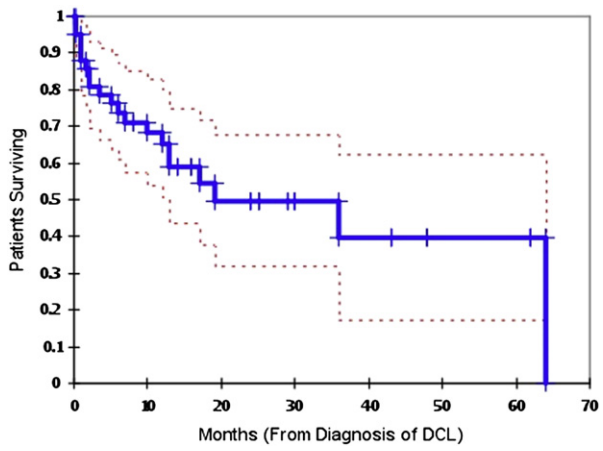

Figure 3. Kaplan-Meier curve depicting overall survival (OS) in all reported cases of donor cell leukemia (DCL). Overall, at least 34 patients had died before publication, at a median of 5.5 months after DCL diagnosis. At least 24 patients remained alive at publication, at a median of 14 months follow-up. Reinduction chemotherapy with apparently curative intent had been attempted in at least 47 cases. Detailed outcome data were available for 41 patients. Median OS for treated patients was estimated at 32.8 months (95% confidence interval: 22.5-43.1 months). For the Kaplan-Meier estimate, surviving patients were censored at last reported follow-up. Insufficient data were provided for analysis of event-free survival (EFS) or other detailed outcome parameters.

(because "last/latest follow-up" ranged from 24 to 66 months). Thus, durable remissions can be obtained with standard reinduction strategies, particularly when consolidated with second HSCT.

Intuitively, an alternative donor would seem preferable for second HSCT, given that significant graft-versus-leukemia (GVL) effect would not be anticipated in the total absence of HLA disparity between donor T cells and the leukemic clone. Intriguingly though, Jacobs et al. (case #62) successfully employed T-replete BMT from the original donor in a patient with donor-AML, achieving CR durable at 4 years [56]. Rationale offered for selecting this "semi-autologous graft" included a low probability of graft rejection while exploiting the unusual opportunity to combine advantages of autologous transplantation (eg, lack of GVHD) with a graft free of disease contamination. The authors suggested that even in this setting, "GVL reactions may occur." Although identity of donors was often not stated, at least 4 other patients also received a second HSCT from their original donor. Overall, 3 (of the 5) achieved durable responses, supporting Jacobs and coworkers' suggestion that "second transplant from the same donor may be a curative option for these patients." Equally surprising were the transient responses to donor lymphocyte infusions (DLI) from original donors observed in 2 DCM cases (#27 and #30) [36,39]. In the latter, pancytopenia was corrected entirely following DLI for a 12-month period, at which point repeat DLI was unsuccessful. Komrokji et al. hypothesized that acquisition of additional antigens by the donor clone might have made it more recognizable to donor lymphocytes. However, an allogeneic graft-versus-stroma effect on the BM microenvironment (rather than the cells themselves) has been proposed, and could theoretically explain responses observed to both DLI and second HSCT from original donors. Future DCLs might provide unique models for investigating the contribution of cell-independent immunomodulatory properties of allogeneic therapy. For now, however, a second HSCT from an alternative donor would remain a more logical consolidation strategy in DCL.

Ethical Considerations

DCL raises important questions regarding the responsibility of physicians and registries toward donors and their families. Although most sibling donors will be aware of clinical developments in the recipient, stable family relationships should not be assumed, and the situation is clearly different for unrelated donors. Feedback to donor registries of adverse events in recipients is generally encouraged, but although certain registries and cord banks do advocate donor notification in the event of detecting a potentially treatable abnormality, this is rarely a compulsory requirement, and the lack of centralization and international nature of many modern donations can make this practically difficult. Most donor registries have specific policies for sharing information on the recipient's progress with donors, although feedback of adverse outcomes is generally discouraged to prevent misplaced feelings of guilt in the donor. Nevertheless, many transplant centers will agree to provide donors with progress updates, or even permit limited communication with the recipient provided both parties have consented. However, how this applies to DCL is unclear because abnormalities are usually undetectable in donor samples pretransplant, and the clinical implications for the donor remain unclear. Ethically, there is a strong argument in favor of notifying a donor when their HSCs become malignant in another host. Donor tracing would additionally yield useful follow-up information on the health of the donor and potentially also on relevant pathology samples to aid further research into DCL leukemogenesis. Conversely, DCL is clearly multifactorial, and current evidence suggests that donors are not destined to develop malignancy themselves. The EBMT survey inquired about donor health and revealed no cases of leukemia in donors from the 14 reported cases, at a median follow-up of 9 years [6]. BM and/or cytogenetic examination were performed in 4 of the donors, with no abnormalities detected. This larger cohort similarly yielded no reports of donors developing leukemia.

Consequently, it is difficult to advocate detailed notification and assessment of unrelated donors on the basis of risk, and anything more than a simple health surveillance interview would be difficult to justify given the potential negative impacts of such information on a healthy individual's mindset, relationships, insurance status, etc. For now, an ad hoc discretionary approach seems reasonable, based on local policy and the known wishes of the donors/recipients involved. The duties of cord banks toward donors and their parents are particularly difficult to define, given the unusually high frequency of preleukemic clones demonstrable in CB samples and the particular sensitivities involved in diagnosing infants with a preleukemic "potential" of doubtful significance. Given that overall risk of the child developing leukemia appears low (<1%), informing the parents would seem unwarranted, although this is clearly open for debate.

CONCLUSION

DCL is an intriguing phenomenon, which was probably underdiagnosed for many years and could represent up to 5% of all leukemia relapses post-HSCT. Etiologic mechanisms remain speculative, but etiology is almost certainly multifactorial, and

analysis of the reports to date reveals a marked degree of heterogeneity between cases. New molecular techniques for chimerism monitoring have elevated the ease, applicability, and reliability of DCL diagnosis to unprecedented levels, and it is perhaps not surprising that examples of DCL in the literature have exploded in parallel. These modern tools are a far cry from the original controversial claims of donor origin made on isolated observations of sex mismatch on karyotype analysis—some of which may have been erroneous and could be serving to confound/confuse analysis of the cohort collated herein. It is clear, though, that DCL as an entity exists and should be considered in any cases of acute leukemia developing in the posttransplant period, particularly if features differ from those of the original disease. With growing awareness and increasingly sophisticated diagnostic tools, it should become feasible to report larger series, with a view to identifying risk factors and optimal treatment strategies. A greater understanding of the biology and impact of DCL will raise other issues regarding donor recruitment, selection, and notification, particularly in the context of increasing age of potential donors and rising popularity of CBT. Future cases should also provide valuable human models for investigating mechanisms of leukemogenesis in vivo.

ACKNOWLEDGMENTS

Financial disclosure: The authors have nothing to disclose.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.bbmt.2010.10.010.

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